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14. ABSTRACT Electrodes modified with bilayers that incorporate cytochrome c oxidase (CCO), the terminal enzyme in mammalian respiration, will be studied as biosensors for cyanide. This CCO modified electrode has an architecture that exhibits robust response behavior and stability that mimics the <i>in vivo</i> behavior of this enzyme. These CCO modified electrodes remain active on storage in buffer, can withstand exposure to temperatures as extreme as 800 C (1760 F) and have a functional lifetime exceeding two months. The structure of the CCO modified electrode proposed for study here is uniquely similar to its <i>in vivo</i> environment in the inner mitochondrial membrane. No other enzyme modified electrodes reported thus far in the literature has this structure. Experiments have shown that the electrochemical response of these CO modified electrodes to the oxidation of reduced cytochrome c (its reductive reaction partner) is sensitive to cyanide and the response is reversible. Work proposed here will characterize the affect of cyanide on the direct electron transfer reaction of these CCO modified electrode with ambient dioxygen concentrations (its oxidative reaction partner). Initial experiments testing this hypothesis have been positive. This is a simpler biosensor configuration compared with the cytochrome c system described above (no added component) and it has potential for providing a practical sensors with failure to military applications for toxins that inhibit the electron transfer reactions of CCO with lethal consequences.				
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INTRODUCTION

The main purpose of the current grant was to develop a cytochrome c oxidase (CCO) based biosensor that has value to the United States Armed Forces in the early and remote detection of lethal agents such as cyanide at very low concentrations. According to the project schedule for the first year we've investigated successfully the dependence of steady state current for electroreduction of oxygen on cyanide concentration, the applied overpotential and temperature, and effect of endogenous components such as carbon monoxide, nitric oxidase, ascorbate, azide, sulfide affecting on the CCO reactivity. The results suggest that the CCO modified electrode can be used for sensing cyanide. However, in order to develop a practical biosensor for cyanide under conditions of interest to the United States Army, stability of CCO modified electrode's response to cyanide and effects of serum albumin on the response of CCO to cyanide will be investigated. CCO modified gold disk electrode will be prepared without guiding from the QCM data and evaluated with CV.

BODY

The current response for the electroreduction of oxygen in solution on the cytochrome c oxidase (CCO) modified electrodes was investigated while introducing samples of cyanide under flow injection conditions (FIA) without cytochrome c. Figure 1 shows sequential FIA of the 50 μ l, 50 μ M sodium cyanide on the CCO modified electrode. Once blank buffer was introduced into the electrochemical cell, the stable reductive current was observed in the beginning. When 50 μ l 0.1 M phosphate buffer (pH=7.4) containing 50 μ M cyanide was introduced into the cell, the decrease in the reductive current was observed initially following by the corresponding current plateau. The change in the reductive current for the sequential injection of cyanide is nearly the same. The responses shown in Figure 1 are representative of the responses obtained at approximately 5 different CCO modified electrodes.

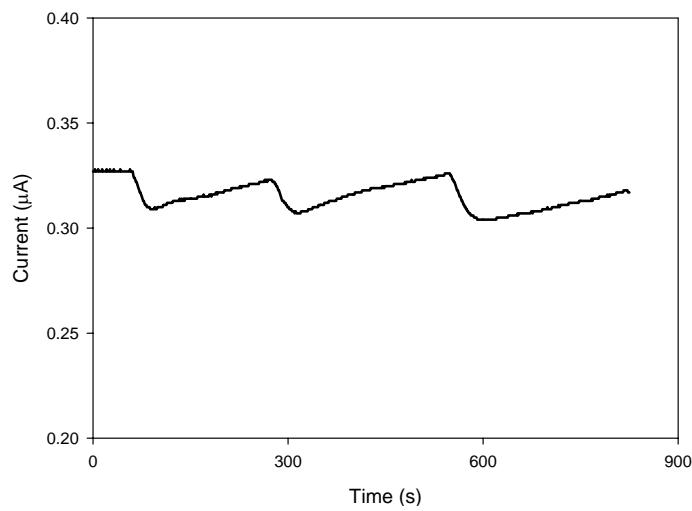


Figure 1, FIA experiment on the oxidase modified electrode at the flow rate of 500 μ l/min. The potential of the working electrode was held at -0.20 V vs Ag/AgCl. 50 μ l phosphate buffer (0.1 M, pH = 7.4) containing 50 μ M cyanide was injected into the cell.

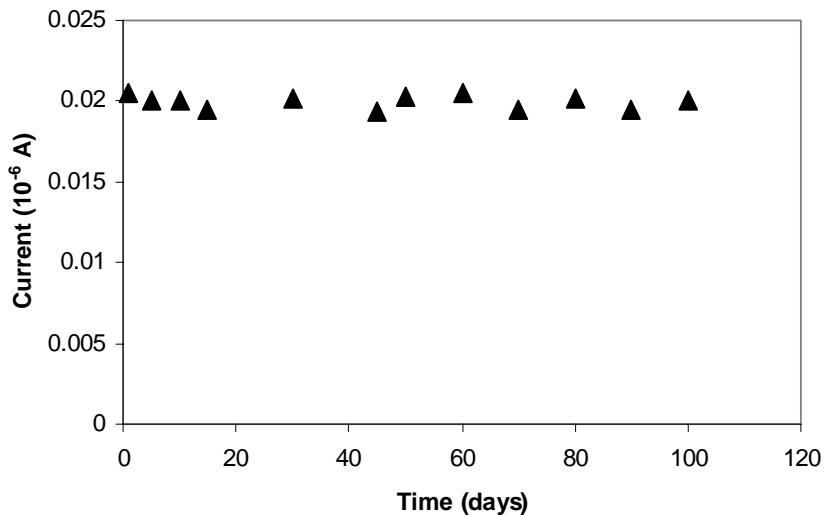


Figure 2, Current dependence upon time. Flow rate: 500 μ l/min; -0.20 V vs Ag/AgCl; 50 μ l phosphate buffer (0.1 M, pH = 7.4) containing 50 μ M cyanide was injected into the cell.

The stability of the oxidase modified electrode was tested by storing the modified electrode in the refrigerator at 4 °C. As shown in Figure 2 (solid triangle) there was no apparent decrease in the response to 50 μ M cyanide within 100 days when storing at 4 °C in air. The stability demonstrated that the oxidase modified electrode retained a biomimetic environment to maintain the activity of the cytochrome c oxidase. The oxidase modified electrode can be stored at 4 °C in air for practical application. However, when continuously soaking the modified electrode in 0.1 M phosphate buffer (pH=7.4) at 4 °C, the current response to 50 μ M cyanide decreased quickly within 2 days.

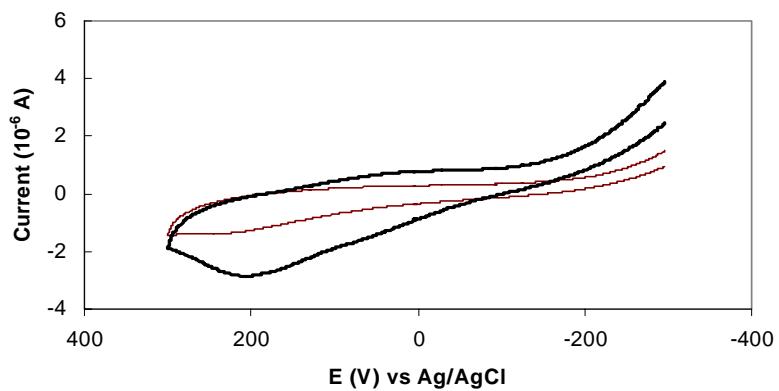


Figure 3, cyclic voltammograms on the oxidase modified electrode at the scan rate of 20 mV/s in 0.1M phosphate buffer (pH=7.4) without (bold line) and in the presence of 0.8% serum albumin.

Serum albumin can strongly adsorb to the electrode interface and may foul the oxidase modified electrode. A typical cyclic voltammograms on the oxidase modified electrode in 0.1 M phosphate buffer (pH=7.4) without (bold line) and in the presence of 0.8% serum albumin is

shown in Figure 3. The magnitude of the oxidative peak current in the phosphate buffer solution containing albumin is much smaller than that without albumin. A typical flow injection analysis experiment on the oxidase modified electrode in 0.1 M phosphate buffer (pH=7.4) without (bold line) and in the presence of 0.8% serum albumin is shown in figure 4. The change in magnitude of the current increases as the concentration of cyanide increases.

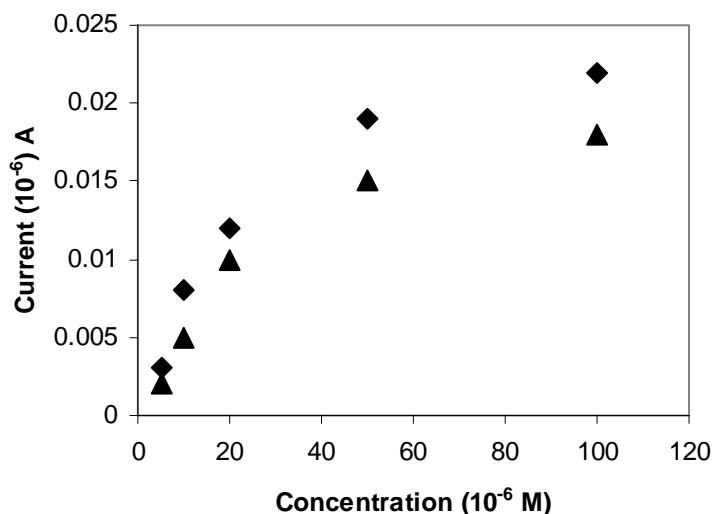


Figure 4, magnitude of the current change as a function of cyanide without (square) and in the presence of 0.8% serum albumin (triangle). Flow rate: 500 μ l/min; -0.20 V vs Ag/AgCl; 50 μ l phosphate buffer (0.1 M, pH = 7.4) containing cyanide was injected into the cell.

The electroreduction of oxygen dependence on cyanide concentration in the presence of 0.8% serum albumin is similar to that without serum albumin. The responses shown in figure 4 are representative of the responses obtained at approximately 5 different CCO modified electrodes. The oxidase modified electrode is shown to be quite robust.

Octadecyl mercapton (OM) was deposited onto Au disk electrode without Quartz crystal microbalance monitoring. The OM modification of the Au electrode for electrochemistry makes it possible to generate barrier layer that prevents free diffusion of electroactive species to the surface of the electrode. Since $\text{Fe}(\text{CN})_6^{3-}$ represents a convenient and electrochemical reversible, one electron, outer-sphere redox couple, it was selected as an electrochemical probe for the coverage of OM submonolayer. The typical cyclic voltammogram of the gold electrode before and after deposition of mercapton submonolayer in 5 mM ferricyanide containing 0.1 M KNO_3 is shown in figure 5. A pair of reversible redox peaks on the gold electrode without and with mercapton submonolayer was observed, respectively. But the magnitude of peak current on the gold electrode with mercapton submonolayer is about half as much as that without mercapton submonolayer, which demonstrates that mercapton submonolayer has been formed on the surface of the gold electrode to insulate the gold electrode. Such insulation reduces redox reaction $\text{K}_3[\text{Fe}(\text{CN})_6]$ that occurs on the surface of the gold electrode with the partly coated mercapton.

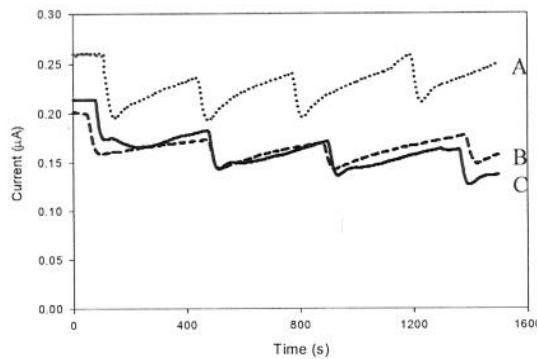


Figure 5, FIA experiment on the CCO modified electrode. Flow rate: 0.5 ml/min; -0.25 V vs Ag/AgCl; Cyanide volume injected: 50 μ l; 50 μ M cyanide. A: 40 $^{\circ}$ C; B and C: 21 $^{\circ}$ C.

The typical cyclic voltammograms on the oxidase modified electrode in 0.1 M phosphate (pH=7.4) at different scan rates are shown in Figure 6. A pair of redox peaks at each scan rate was observed. The peak to peak separations are too large for an ideal reversible electrochemical process arising from the adsorbed cytochrome oxidase and the voltammetric peaks are not symmetrical. No redox response of a lipid modified electrode without cytochrome c oxidase was observed.

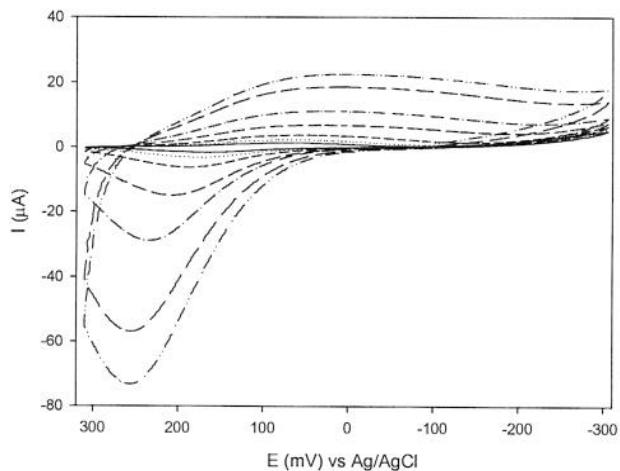


Figure 6, the typical cyclic voltammograms on the gold electrode in 5 mM

Since the rate of OM self-assembly onto the gold depends on the thiol concentration, mass transfer (i.e., agitation) and surface property of gold electrode, the amount of OM self-assembled onto the surface of a gold electrode is not easily controlled without in-situ monitoring. By controlling deposition time of OM, just only one of five electrodes has above the results. Deposition of OM onto gold disk electrode can be monitored with impedance and spectroscopy. It may take more time to solve the problem for the oxidase electrode preparation. However, the dependence of the current response for oxygen reduction on cyanide concentration and stability and reversibility for the sequential flow injection analysis of cyanide on the cytochrome c oxidase electrode is in agreement with the previous results on the oxidase electrode prepared with EQCM.

KEY RESEARCH ACCOMPLISHMENTS

The tasks proposed in the Statement of work for this project have been successfully met during the final year.

Task 3.

- After stored at 4 °C in air about 100 days, the oxidase modified electrode has no apparent decrease in the response to cyanide (see Figure 2). The stability demonstrated that the oxidase modified electrode retained a biomimetic environment to maintain the activity of the cytochrome c oxidase. The oxidase modified electrode can be stored at 4 °C in air for practical application.
- Serum albumin can strongly adsorb to the electrode interface and may foul the oxidase modified electrode but the affect on detection of cyanide in flowing injection analysis is very small (see Figure 3, 4).

Task 4.

- Octadecyl mercapton (OM) was deposited successfully onto Au electrode without Quartz crystal microbalance monitoring to prepare the oxidase electrode. (see Figures 5, 6)

CONCLUSION

According to the project schedule for the final year we have successfully investigated stability of CCO modified electrode's response to cyanide and effects of serum albumin on the response of CCO to cyanide, prepared successfully CCO modified gold disk electrode without guiding from the QCM data and evaluated the electrode with CV. The results suggest that the CCO modified electrode can be used for sensing cyanide. The analytical device could be used under conditions of interest to the United States Army.